ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS), BAP-TOXICITY AND BAP-MUTAGENIC EQUIVALENTS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) TO NIGERIAN-PETROLEUM PRODUCTS

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ABSTRACT
The 96-h LC_{50} acute toxicity and PAH levels in 720 fingerlings (0.9±0.01g) of Clarias gariepinus to various triplicate doses 1, 3, 6, 9, 12 and 0.0 ml/L (control) of crude oil, petrol, kerosene and diesel. There was no mortality in the control but 10, 20, 50 and 60% occurred in crude oil; 10, 20, 50 and 70% in petrol; 10, 20, 50 and 70% in kerosene and 10, 20, 30, 40 and 60% in diesel respectively at 1, 3, 6, 9 and 12 ml/L after 96 hour exposure. The logarithmic probit lines and R^2: y = 1.372x + 3.724, R^2 = 0.997; y = 1.665x + 3.694, R^2 = 0.998; y = 1.665x + 3.694, R^2 = 0.998 and y = 1.665x + 3.694, R^2 = 0.998 and 96-h LC_{50}: 9.6, 6.98, 6.2 and 11.0ml/L represented toxicity values for crude oil, petrol, kerosene and diesel to C. gariepinus. Total pah value of petroleum products in exposed fish ranged from highest value of 99.68±4.81 crude oil > 97.30± 14.57 diesel oil > 35.413±0.90 petrol > 32.72±3.60 ng/µL kerosene. The lowest value of 0.061 was shown in Nap while highest value was 384.68 in DahA >383.47 B[ghi] P > 361.38 IP >236.41BaA >71.59 BbF>60.17 BkF >37.17 BaP> 22.82FI> 17.72A >5.33Chry >4.56 Flu> 4.35 Phe>2.79 Acy>2.40Ace >0.06Nap for crude oil. The sequence in petrol indicate highest value of 95.45BaA >94.50 IP>89.61 B[ghi] P >83.67 DahA>72.46 BkF>50.65BbF >44.69 BaP>13.19 FI>11.57 Chry>5.67 A>2.78 Phe>2.56Flu for petrol. In kerosene, the sequence from highest level of 91.56 BaA>90.69 IP> 77.96 BkF>75.99 B[ghi] P >49.55 BbF>41.31 BaP>11.34 FI>9.11 Chry>6.63 A>2.48 Phe>1.97Flu. Diesel oil however indicated highest value of 389.05DahA> 355.70 B[ghi] P >343.48 IP>262.80 BaA>65.35 BbF>58.16 BkF>30.38 BaP>20.09 FI>18.48 A>4.79 Chry>3.79 Phe>2.51 Acy>2.22. Mean ∑ 16PAH of lighter products showed greater toxicity than heavier products due to greater level of BaA - BaP compared to DahA- BaP. On the other hand, heavier petroleum with greater total mean ∑ 8PAH was more carcinogenic and mutagenic compared to lighter petroleum.
Key words: Petroleum, Toxic equivalent factor, Mutagenic equivalent factor

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are group of compounds consisting of two or more fused aromatic rings which are formed during incomplete combustion of organic materials such as wood and fossil fuels, petroleum products and coal (Li and Leu, 2001) . They are ubiquitous pollutants frequently found in a variety of environments such as freshwater marine sediments, atmosphere and ice (Choi et al., 2010). Many PAHs and their expoxides are highly toxic to microorganisms, fishes and man (Ramesh et al., 2011; Isioma *et. al.*, 2017). In recent times, PAHs have received much attention due to their potential cause of cancer, mutagenic disorders and birth defects (Martinez et al., 2004; Ramesh et al., 2011). Adverse effects of PAHs have also been observed in marine organisms and include growth reduction, endocrine alteration, and malformation of embryo and DNA damage (Lawal, 2017). Ingestion of contaminated food and diffusion from water across the gills and skin are major routes of PAHs exposure to fish. Due to the lipophilic nature and high chemical stability of PAHs, they accumulate in the fatty tissues of fish following their uptake. Fishes are therefore good indicators of pollution in inland and coastal waters (Li et al., 2011; Ezike et al., 2017). Two broad groups exist based on their physical and biological properties including, high molecular weight (HMW) and low molecular weight (LMW) PAHs. The HMW PAHs consists of 4-6 aromatic rings and are less readily bio-degraded by indigenous microorganisms, hence can persist in the aqueous environment by bio-accumulating in aquatic organisms like fish and mussels and are more carcinogenic. The LMW PAHs consists of 2-3 aromatic rings and is less carcinogenic. However, it poses toxic effect to many aquatic organisms (Brown and Peake, 2006). The concentrations of petroleum products toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved. (Lonning, 1977). Estimated concentrations of petroleum toxic to fish eggs and fingerlings to be 0.5-10 mg/L Benzo (a) pyrene binds to DNA to cause cancer and is frequently used as a marker for carcinogenic disorders and may provide the basis for predicting the impact of exposures of PAH to *C.gariepinus* fingerlings (Payne et al., 1975).

Although fishes have oxidative enzymes for metabolic detoxification of xenobiotics including aromatic petroleum hydrocarbons (Payne et al., 1975). Little is known about the PAH level of petroleum in exposed fish. The uptake and translocation of crude oil and other petroleum products /or compounds in fish may be the gills, guts or the intestinal wall (Roubal et al., 1977). The parent compounds readily solublize in cell membranes and are probably carried via the

erythrocytes to the general circulation of the blood. Some of the compounds may be carried by lipoproteins and leukocytes in the blood to the liver. The major route of excretion of petroleum metabolites is through the bile; into the intestine and out with faeces. Some are excreted through the gills and kidney (Lee, 1976).

BaP-TEQ (carcinogenic equivalents and BaP- MEQ (mutagenic equivalents are measure for sum of total 8 number of particulate PAHs (∑8PAH), having molecular weight greater than 228 gram. ∑8PAH includes benzo (a) pyrene (BaP), benz (a) anthracene (BaA), chrysene/iso-chrysene (CHR), benz (b) fluoranthene (BbF), benzo (k) fluoranthene (BkF), indo (123-cd) pyrene (IP), Dibenz (a,h) anthracene (DahA) and benzo(ghi) pyrelene (BghiP) (Nisbet and Lagoy, 1992; Durant et al., 1996).

The African catfish of genus *Clarias* are esteemed group of fish with high market value in Tropical Africa (Reed et al., 1967). Their hardy nature and possession of accessory air-breathing organs enable them to tolerate adverse aquatic conditions. Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil and other petroleum products. This study was undertaken to determine the comparative toxic level of PAH of various petroleum products on *C. gariepinus* fingerlings

MATERIALS AND METHODS

Experimental fish and petroleum

A total of seven hundred and twenty (720) fingerlings of African catfish (mean weight 0.96 ± 0.1g) were obtained from local outskirts in Enugu Nigeria and transported to Fisheries Laboratory of the Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology ESUT, Enugu Nigeria. They were held in four fiber reinforced plastic (FRP) tanks, containing 320 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for two weeks and were fed with commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Petroleum (crude oil, petrol, kerosene and diesel) was obtained from Nigerian National Petroleum Cooperation Enugu. The water soluble fraction WSF was prepared following the method of UNEP (1989), which involved 20-h mixing of 10:1 clean water to petroleum with a rotator magnetic stirring rod, separated layers after resting for 12-hrs with separating flask before storing as stock solution in corked 50L plastic gallons. Ethical clearance from Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

Acute toxicity test

Toxicity of petroleum to *C. gariepinus* was carried out according to the OECD guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200 L capacity glass aquaria. Thirty (30) fish per treatment were randomly exposed to 6 experimental treatments (1, 3, 6, 9, 12 and 0 ml/L of water soluble fractions WSF which served as the control of each triplicate group of petroleum product (crude oil, petrol, kerosene and diesel), to determine 96h LC_{50} using the probit analysis proposed by Finney (1971) and polycyclic aromatic hydrocarbons (PAH) in exposed fish (Lonning, 1977). The exposure pollutant was renewed each day and was analyzed using LC–MS/MS to ensure the agreement between nominal and actual concentrations of the petroleum in the aquaria [9]. The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analyzed daily, using standard methods (APHA, 2005) and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1} , temperature 27.75 ± 0.5 ^{0}C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}). The test fish of 9 and 12 ml/L in each product were sampled to determine ∑16PAH, ∑8PAH, TEQ and MEQ of each product. A portion of each sample using the GC-MC was taken for extraction and analysis of PAH (Takatsuki et al., 1985; Neff, 1985).

PAH extraction

The method described by Takatsuki et al. (1985) with slight modification for extraction and dosing of PAHs was employed. known portion of fish tissue was introduced into a round bottomed flask containing 200 ml of ethanol, 35 ml of 50% aqueous KOH and 2 g of $Na_2S_9H_2O$ (sodium sulfide monohydrate). The mixture was refluxed on a hot plate for two hours before cooling and maintaining at 40 °C. 100 ml of n-hexane was added in the portion with a slight swirling to allow homogenization. The mixture was rested and separated in a separating funnel. The solution was then extracted, filtered and collected through anhydrous sodium sulphate to a final volume of 3 to 5 ml using a rotary evaporator. The chromatographic column (20 mm ID) was dried using 8 g of silica gel and 3 g of anhydrous sodium sulphate to cover the silicate gel. The chromatographic columns were then washed with 30 ml of n-hexane and eluted and poured to the top level of the column. The columns were covered with aluminum foil and the concentrated solution containing the PAHs was introduced and rinsed three times with 2 ml of n-hexane. The eluted solution was evaporated to 1 : 2 ml of solution while the residual solvent was evaporated under nitrogen, followed by dissolution of the PAHs in 1 ml of acetonitrile and injected into the HPLC-FID. To quantify the toxicity or carcinogenic potency of PAHs relative to BaP, the

toxic equivalent factors (BaP_{TEF}) and mutagenic equivalent factors (BaP_{MEF}) relating the carcinogenic mutagenic potency of individual PAH to BaP have been used (Nisbet and Lagoy, 1992; Durant et al., 1996). The BaP carcinogenic equivalent (BaP_{TEF}) and BaP mutagenic equivalent (BaP_{MEQ}) for the individual PAHs was calculated: $BAP_{TEQ} = \sum C_1 \text{ X } BAP_{TEF}$; $BAP_{MEQ} = \sum C_1 \text{ X } BAP_{MEF}$, where Ci = concentration of PAHs.

The equations for measurement of BaP-TEQ and BaP-MEQ are given below.
(BaP-TEQ)Σ8PAH = [BaA] × 0.1 + [CHR] × 0.01 + [BbF] × 0.1 + [BkF] × 0.1 + [BaP] × 1 + [IcdP] × 0.1 + [DahA] × 5 + [BghiP] × 0.01. (BaP-MEQ)Σ8PAH = [BaA] × 0.082 + [CHR] × 0.017 + [BbFA] × 0.25 + [BkFA] × 0.11 + [BaP] × 1 + [IcdP] × 0.31 + [DahA] × 0.29 + [BghiP] × 0.19 [5, 17].

Statistical Analysis

Data obtained were expressed as standard mean ± standard error of mean and analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago Illinois, USA). Differences in the test 16PAHs of various products were subjected to one way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine level of difference at 95% probability level. Pearson correlation was used to determine relationship between various petroleum products and regression of their 8PAHs.

RESULTS

Table 1: Exposure of C. gariepinus to acute concentrations of petroleum for 96 hours

Petroleum	Conc. ml/L	Log conc.	Period (hr)				Percentage mortality	Probit Mortality
			24	48	72	96		
Crude oil	0	0	0	0	0	0	0	0
	1	0				1	10	3.72
	3	0.30			1	1	20	4.16
	6	0.78		1	1	2	40	4.76
	9	0.95		1	1	3	50	5.00
	12	1.08	1	1	2	2	60	5.25
petrol	0	0					0	0
	1	0				1	10	3.72
	3	0.30				2	20	4.16
	6	0.78		1	1	3	50	5.00
	9	0.95	1	1	2	2	60	5.25
	12	1.08	1	1	2	3	70	5.52
kerosene	0	0					0	0
	1	0				1	10	3.72
	3	0.30			1	1	20	4.16
	6	0.78	1	1	1	2	50	5.00
	9	0.95	1	1	2	2	60	5.25
	12	1.08	1	1	2	3	70	5.52
Diesel	0	0					0	0
	1	0				1	10	3.72
	3	0.30			1	1	20	4.16
	6	0.78			1	2	30	4.48
	9	0.95		1	1	2	40	4.76
	12	1.08		1	2	3	60	5.25

Mortality and Logarithmic probit line for 96-h LC_{50} of petroleum products to C. gariepinus

Table 1 gave mortality values of fish to various concentrations (0, 1, 3, 6, 9 and 12 ml/L. There was no mortality in the control but 10, 20, 50 and 60% for crude oil; 10, 20, 50 and 70% for petrol; 10, 20, 50 and 70% for kerosene and 10, 20, 30, 40 and 60% for diesel occurred respectively in 1, 3, 6, 9 and 12 ml/L after 96 hour exposure. Figure 1-4 gave the logarithmic probit lines and R^2: y = 1.372x + 3.724 , R^2 = 0.997; y = 1.665x + 3.694, R^2 = 0.998 ; y = 1.665x + 3.694, R^2 = 0.998 and y = 1.665x + 3.694, R^2 = 0.998 and 96-h LC_{50}: 9.6, 6.98, 6.2 and 11.0ml/L for crude oil, petrol, kerosene and diesel to *C. gariepinus*

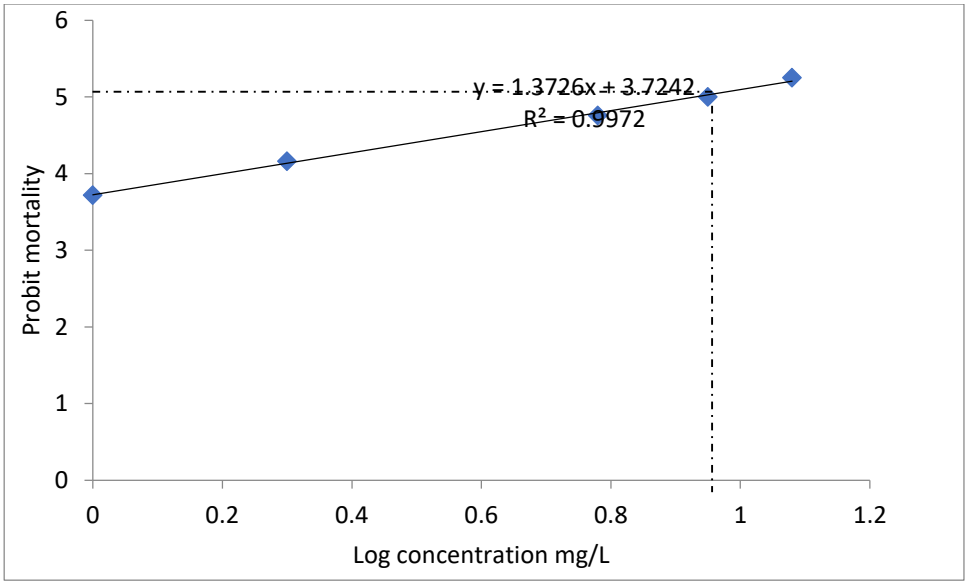

Figure 1: Logarithimic probit line for determination of 96-h LC_{50} crude oil to *C. gariepinus*

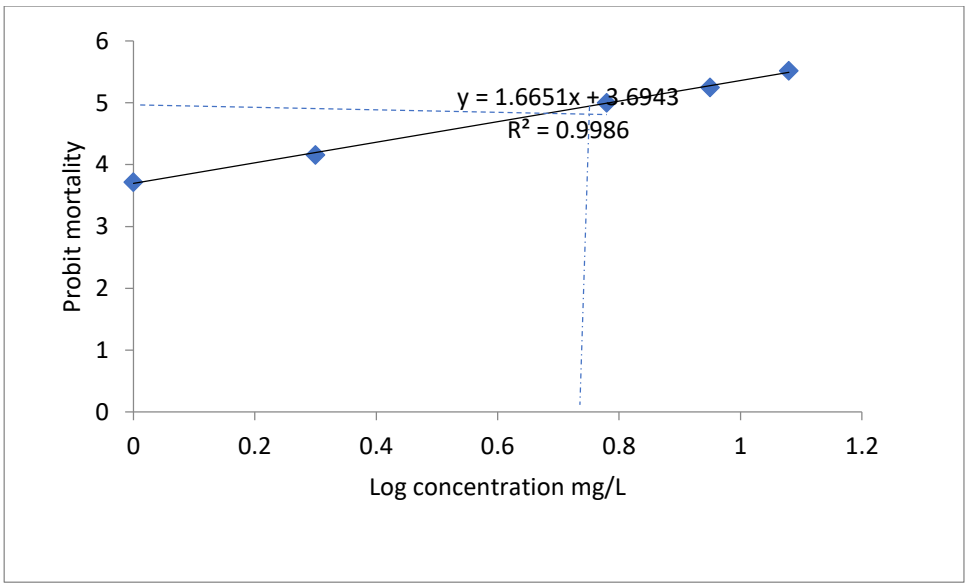

Figure 2: Logarithimic probit line for determination of 96-h LC_{50} petrol to *C. gariepinus*

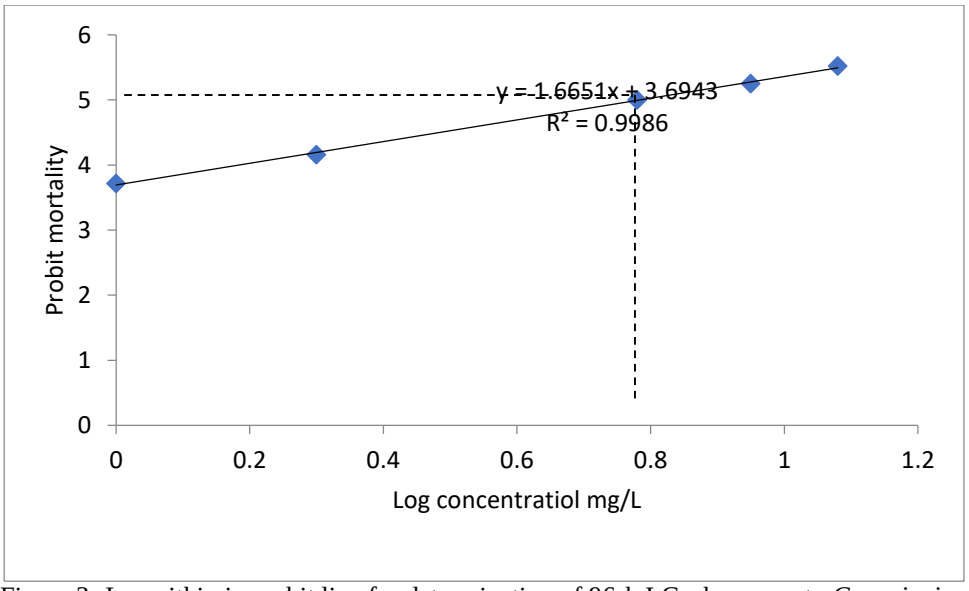

Figure 3**:** Logarithimic probit line for determination of 96-h LC_{50} kerosene to *C. gariepinus*

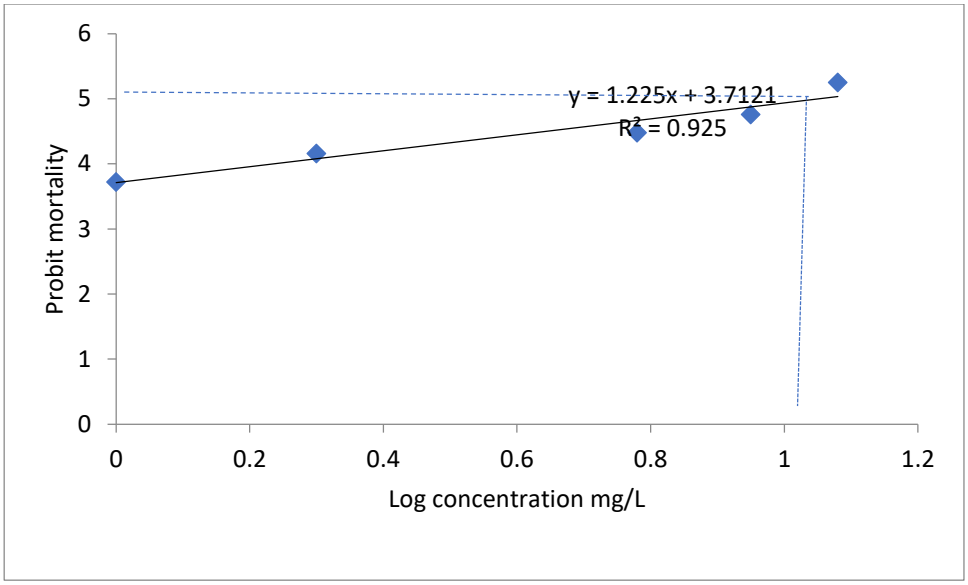

Figure 4**:** Logarithimic probit line for determination of 96-h LC_{50} Diesel to *C. gariepinus*

Table 2: PAH in fish exposed to Petroleum

Pp	C	PAH ng/µL																
		Nap	Acy	Ace	Flu	Phe	A	FI	P	BaA	Chry	BbF	BkF	BaP	DahA	IP	BghiP	Total
CO	9	0.06± 0.0003^{a}	2.79± 0.0006^{c}	2.40± 0.0003^{b}	4.56± 0.0006^{e}	4.35± 0.0006^{d}	17.7237± 0.0003^{f}	22.82±0 .0006^{g}	nd	236.41± .0006^{k}	5.3280± .0006^{e}	71.58± 0.0003^{j}	60.16± .0057^{i}	37.11± .0006^{h}	384.68^{n}	361.37± .0006^{l}	383.47± .0003^{m}	99.6813± 4.81437
	12	0.06± 0.0010^{a}	2.79± 0.0008^{c}	2.40± 0.0003^{b}	4.58± 0.0113^{e}	4.35± .0003^{d}	17.72±0 .0009^{f}	22.82±0 .0009^{g}	nd	236.44± .0282^{k}	5.33± .0038^{e}	71.59± .0032^{j}	60.17± .0033^{i}	37.17± .0306^{h}	384.69± .0067^{n}	361.39± .0069^{l}	383.48± .0103^{m}	
P.	9	Nd	Nd	nd	2.55± 0.0003^{a}	2.78b	5.69± .0100^{c}	13.19± .0006^{e}	nd	95.14± .0006^{l}	11.57± .0006^{d}	50.65± .0003^{g}	72.45± .0007^{h}	44.69± .0003^{f}	83.67± .0003^{i}	94.50± .0057^{k}	83.67± .0003^{j}	35.40713± .90029
	12	Nd	Nd	nd	2.56± 0.0012^{a}	2.79± .0009^{b}	5.69± .0100^{c}	13.19± .0032^{e}	nd	95.15± .0006^{l}	11.57± .0006	50.65± .0003^{g}	72.45± .0007h	44.69± .0003	83.67± .0003^{i}	94.50± .0058^{k}	89.61± .0048^{j}	
K	9	nd	Nd	nd	1.97± 0.0003^{a}	2.48± .0003^{b}	6.63± .0005^{c}	11.34± .0003^{e}	Nd	72.45± .0005l	9.11± .0006^{d}	49.52± .0298^{g}	77.97± .0006^{i}	41.30± .0058^{f}	84.10± .0003^{j}	90.69± .0003^{k}	75.99± .0026^{h}	32.72± 3.5993
	12	nd	Nd	nd	1.96± 0.0003^{a}	2.48± .0009^{b}	6.63± .0014^{c}	11.38± .0210^{e}	nd	72.46± .0006 ^{l}	9.11± .0035 ^{d}	49.52± .0284^{g}	77.9± .0012^{i}	41.31± .01 45^{f}	84.11± .0009^{j}	90.69± .0023^{k}	75.99± .0026^{h}	
.D.	9	Nd	2.51± 0.0003^{b}	2.22± 0.0006^{a}	Nd	3.79± .0015^{c}	18.48± .0009^{e}	20.09± .0006^{f}	Nd	262.80± .0035^{j}	4.79± .0003^{d}	65.35± .0005^{i}	58.16± .0006^{h}	30.37± .0006^{g}	389.05± .0006^{m}	343.49± .0057^{k}	355.6967± .00067^{l}	97.30± 14.5733
	12	nd	2.51± 0.0009^{b}	2.23± 0.0015^{a}	nd	3.793± .0015^{c}	18.49± .0060^{e}	20.09± .0012^{f}	nd	262.81± .0088^{j}	4.81± .00700^{d}	65.36± .0009^{i}	58.16± .0009^{h}	30.37± .0003^{g}	389.05± .0008^{m}	343.49± .0033^{k}	355.69± .0007^{l}	

Different superscripts in a row indicate significant difference between means (ANOVA, P< 0.05)
KEY: Nap = Naphthalene, Acy= Acenaphthylene, Ace= Acenaphthene, Flu= Fluorene, Phe= Phenathrene, A =Anthracene, FI =Fluoranthene, P = Pyrene, BaA=Benz {a} anthracene, Chry = Chrysene, BbF= Benzo [b] fluoranthene, BkF=Benzo [k] fluoranthene, BaP = Benzo, [a] pyreneDahA = Dibenz [ah] anthracene, IP = Indeno [123-cd] pyrene,, B[ghi]P = Benzo [ghi] pyrene, CO=Crude oil, P= Petrol, K= kerosene, D= Diesel, pp=petroleum products, c= concentration in ml/L, nd= not detected.

PAHs in exposed fish to petroleum

Total PAHs values in ng/µL (Table 2) of petroleum products in exposed fish ranged from highest value of 99.68±4.81 crude oil > 97.30± 14.57 diesel oil > 35.413±0.90 petrol > 32.72±3.60 ng/µL kerosene.. The lowest value of 0.061 was shown in Nap while highest value was 384.68 in DahA >383.47 B[ghi] P > 361.38 IP >236.41BaA >71.59 BbF>60.17 BkF >37.17 BaP> 22.82FI> 17.72A >5.33Chry >4.56 Flu> 4.35 Phe>2.79 Acy>2.40Ace >0.06Nap for crude oil.but P was not detected. The sequence in petrol indicate highest value of 95.45BaA >94.50 IP>89.61 B[ghi] P >83.67 DahA>72.46 BkF>50.65BbF >44.69 BaP>13.19 FI>11.57 Chry>5.67 A>2.78 Phe>2.56Flu for petrol but Nap, Acy, Ace and P werenot detected. In kerosene, the sequence from highest level of 91.56 BaA>90.69 IP> 77.96 BkF>75.99 B[ghi] P >49.55 BbF>41.31 BaP>11.34 FI>9.11 Chry>6.63 A>2.48 Phe>1.97Flu but. P,Nap, Acy and Ace ere not detected. Diesel oil however indicated highest value of 389.05DahA> 355.70 B[ghi] P >343.48 IP>262.80 BaA>65.35 BbF>58.16 BkF>30.38 BaP>20.09 FI>18.48 A>4.79 Chry>3.79 Phe>2.51 Acy>2.22 Ace. P, Flu and Nap were not detected.

Table 3: Bap-TEQ and Bap- MEQ of petroleum products

8PAH ng/µL				Crude oil			Petrol			Kerosene			Diesel		
	LOD	[a]TEF	[b]MEF		TEQ	MEQ		TEQ	MEQ		TEQ	MEQ		TEQ	MEQ
BaA	0.02	0.1	0.082	236.44	23.64	19.38	95.15	9.51	7.80	72.46	7..24	5.94	262.81	26.28	21.55
Chry	0.02	0.01	0.017	5.33	0.05	0.09	11.57	0.11	0.19	9.11	0.09	0.15	4.81	0.48	0.08
BbF	0.02	0.1	0.25	71.59	7.15	17.59	50.65	5.06	12.65	49.52	4.95	12.3	65.36	6.53	16.34
BkF	0.02	0.1	0.11	60.17	6.01	6.61	72.45	7.24	7.97	77.9	7.79	8.56	58.16	5.81	6.39
BaP	0.02	1	1	37.17	37.17	37.17	44.69	44.69	44.69	41.31	41.31	41.31	30.37	30.37	30.3
DahA	0.02	5	0.31	384.69	1923.45	119.25	83.67	418.35	25.94	84.11	420.55	26.07	389.05	1945.25	8.08
IP	0.02	0.1	0.29	361.39	36.13	104.80	94.50	9.45	27.41	90.69	9.06	26.11	343.49	34.34	99.61
BghiP	0.02	0.01	0.19	383.48	3.83	53.86	89.61	0.89	17.02	75.99	0.75	14.44	355.69	3.55	67.58
Total pahs				**192.53**	254.67	44.84	**67.94**	70.77	17.96	**62.64**	61.47	16.86	**188.84**	256.58	31.24

TEF*: toxic equivalency factors for cancer potency relative to BaP (Nisbet and LaGoy et al. 1992)
MEF*: mutagenic potency factor relative to BaP (Durant et al. 1996 and 1999)

Carcinogenicity and mutagenicity equivalents

The least observed difference LOD of PAH among 8PAHs was 0.02ng/µL Total TEQ and MEQ gave 254.67 and 44.84; 70.77 and 17.96; 61.47 and 16.86; 256.58 and 31.24 respectively for crude oil, petrol, kerosene and diesel respectively. BaP-TEQ in CO ranged from 0.05 in Chry to 1923 in DahA; 0.11 in Chry to 418.35 in DahA; 0.09 in Chry to 420.55 in DahA and 0.48 Chry-1.945 DahA respectively in P, K and D. Similarly, BaP-MEQ ranged 0.09 Chry -119.25 DahA in; 0.19Chry-44.69 BaP; 0.15-41.31BaP in kerosene; 0.08 Chry-67.58 BghiP in diesel

Table 4: Pearson correlation of petroleum products

Petroleum products	Crude	petrol	kerosene	Diesel
Crude				
Petrol	0.819*			
	.013			
Kerosene	.773*	.955**		
	.024	.000		
Diesel	.996**	.833*	.777*	
	.000	.010	.023	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Correlation of petroleum products

Crude oil correlated positively to: petrol (r=0.819, $p < 0.05$); kerosene (r= 0.773, $p < 0.05$) and diesel (r= 0.996, $p < 0.01$). Petrol correlated positively to kerosene r= 995, $p < 0.01$) and diesel (r= 0.833, $p < 0.05$).

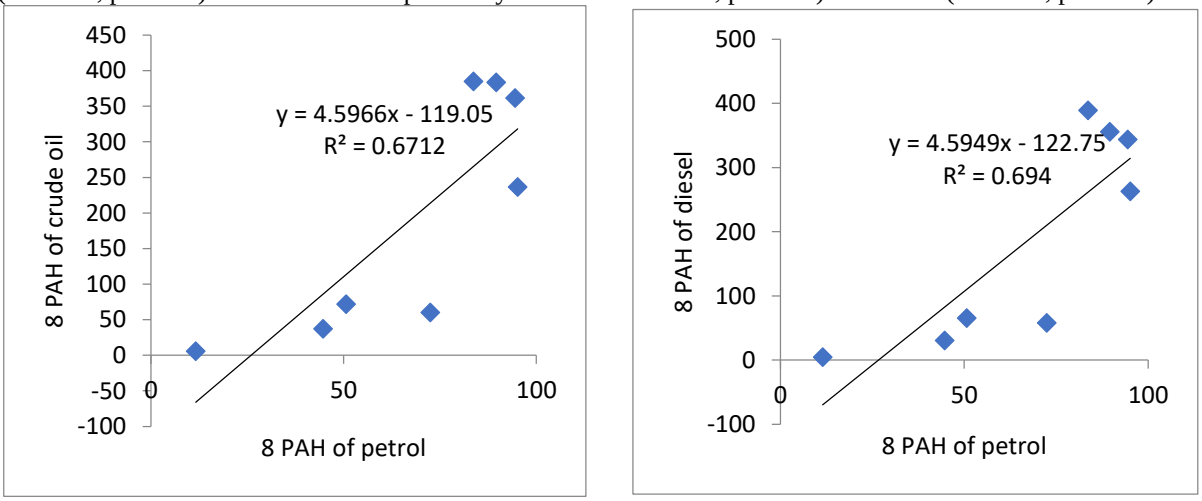

Figure 5: Regression lines of 8 PAH of crude oil / petrol and diesel/petrol

Regression of 8PAHs of petroleum products

The regression lines: y=4.596x - 119.0 gave a value of R^2 = 0.671 when 8PAH of crude oil was compared to petrol, while y=4.594x - 122.7 gave R^2 = 0.694 when diesel was compared to petrol (figure 5).in the same vein y=4.715x102.8, R^2 = 0.597 ; y=4.658x-103.0, R^2 = 0.603 (figure 6) represented regression lines of 8 PAH of crude oil/kerosene and diesel to kerosene respectively. Regression lines of 8 PAH of petrol/kerosene (figure 7) showed that y = 0.878x + 3.069, R^2 = 0.912 and diesel to crude oil (figure 7) indicated y = 0.978x + 0.289, R^2 = 0.991 respectively.

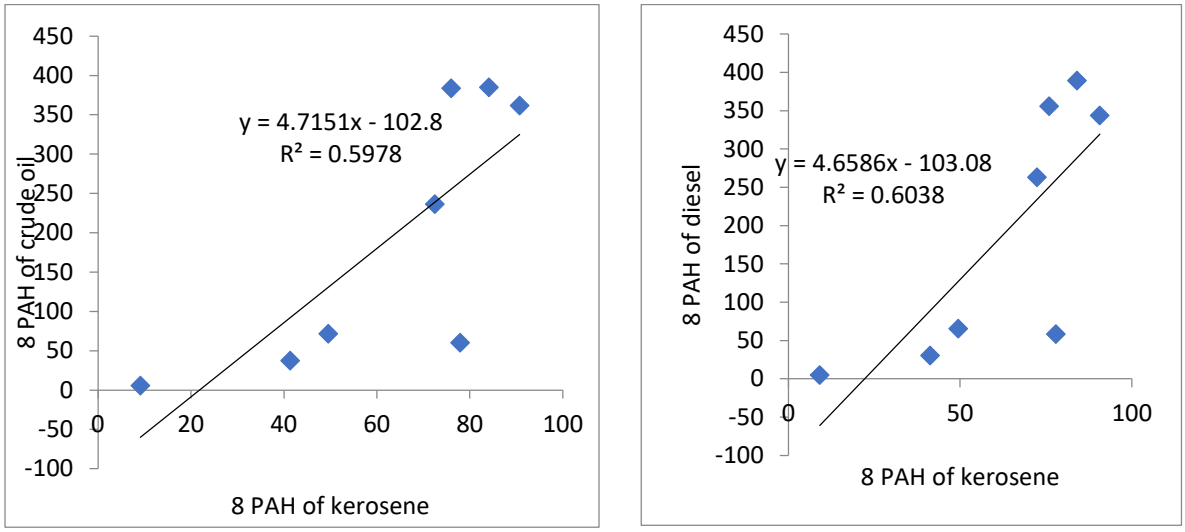

Figure 6: Regression lines of 8 PAH of crude oil/kerosene and diesel to kerosene

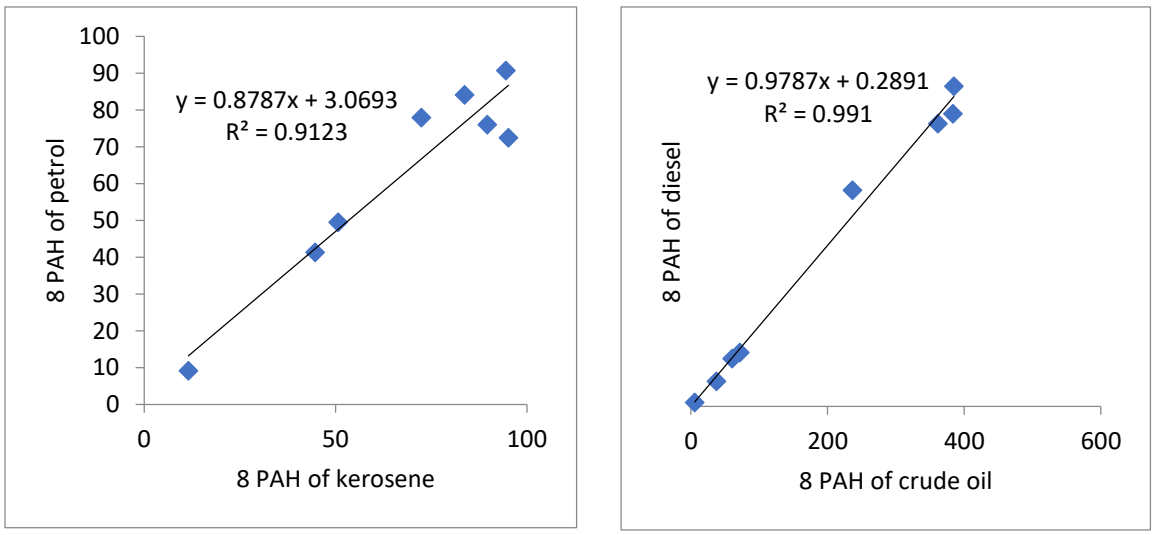

Figure 7: Regression lines of 8 PAH of petrol/kerosene and diesel to crude oil

DISCUSSION

PAHs, Mortality and 96-h LC_{50} of petroleum products to C. gariepinus

In this study PAH values expressed in ng/µL (USEPA, 1997; So – Young et al., 2015) in exposed fish indicated lowest value of 0.061 in Nap and highest value of 384.68 in DahA >383.47 B[ghi]P > 361.38 IP >236.41BaA >71.59 BbF>60.17 BkF >37.17 BaP> 22.82FI> 17.72A >5.33Chry >4.56 Flu> 4.35 Phe>2.79 Acy>2.40Ace >0.06Nap for crude oil but P was not detected. The sequence in petrol indicated highest value of 95.45BaA >94.50 IP>89.61 B[ghi]P >83.67 DahA>72.46 BkF>50.65BbF >44.69 BaP>13.19 FI>11.57 Chry>5.67 A>2.78 Phe>2.56Flu for petrol but Nap, Acy, Ace and P were not detected. In kerosene, the sequence from highest level of 91.56 BaA>90.69 IP> 77.96 BkF>75.99 B[ghi]P >49.55 BbF>41.31 BaP>11.34 FI>9.11 Chry>6.63 A>2.48 Phe>1.97Flu but P,Nap, Acy and Ace were not detected. Diesel oil however indicated highest value of 389.05DahA> 355.70 B[ghi]P >343.48 IP>262.80 BaA>65.35 BbF>58.16 BkF>30.38 BaP>20.09 FI>18.48 A>4.79 Chry>3.79 Phe>2.51 Acy>2.22 Ace. P, Flu and Nap were not detected. This study showed that mortality range of 10 - 70% for petrol and kerosene occurred at concentration range of 1-12 ml/L WSF with 96-h LC_{50} = 6.2 ml/L

petrol and 6.98 ml/L kerosene, while mortality ranged at 10-60% at same dose for crude and diesel with 96-h LC_{50} = 9.6 ml/L crude oil and 11.0 ml/L diesel. A more toxic petrol than kerosene may be due to greater value of 95.45 ng/µL of BaA to 44.69 BaP in petrol than its value of 91.56 ng/µL BaA to 41.31 ng/µL BaP in kerosene. The forgoing gave an indication that lighter petroleum products with lower total mean ∑ 16PAH showed greater toxicity on exposed fish than heavier ones having higher total mean ∑ 16PAH, probably due to greater level of more toxic BaA to BaP compared to less toxic but carcinogenic 384.68 DahA- 37.17 BaP in crude oil and 389.05 DahA- 30.38 ng/µL BaP in diesel (Brown and Peake, 2006). The impact of petroleum water soluble fraction previously under-reported has in recent times posed critical health concerns to aquatic biota, especially fish (Hylland, 2006).

Carcinogenicity and mutagenicity equivalents

Recent approaches has centered to identify and quantify PAHs in water, soil and air environment, their emission sources through various methods in order to evaluate their carcinogenic and mutagenic effects to human health Choi et al., 2010; Peng et al., 2011; Changsheng et al., 2015). The approaches distinguish anthropogenic multiple releases chiefly from petroleum other sources (Payne, 1975; Isioma et al., 2017). BaP is widely accepted as the indicator for measurement of carcinogenicity, thus BaP-equivalent toxicity for other carcinogenic PAHs has been recommended (WHO, 2010) [28] and evaluated for cancer risk assessment (Nisbet and Lagoy, 1992; Durant et al., 1996; Vo Thi et al., 2016).

In our study, BaP-TEQ ranged from 0.05 in Chry to 1923 in DahA with a mean value of 254.67ng/µL for crude oil; 0.11 in Chry to 418.35 in DahA with a mean of 70.77 ng/µL for petrol; 0.09 in Chry to 420.55 in DahA with a mean of 61.47 for kerosene and 0.48 Chry-1.945 DahA (mean =256.58 ng/µL) for diesel. Similarly, BaP-MEQ ranged from 0.09 Chry -119.25 DahA with a mean of 44.84 for crude oil; 0.19 Chry-44.69 BaP with a mean of 17.96 ng/µL in petrol; 0.15-41.31BaP with a mean of 16.86 ng/µL in kerosene; 0.08 Chry-67.58 BghiP (mean = 51.24 ng/µL) in diesel. The foregoing gave an indication that heavier petroleum products with greater mean ∑ 8PAH were more carcinogenic and mutagenic compared to lighter petroleum with lower mean ∑ 8PAH. Crude and diesel oils have shown greater ability than petrol and kerosene to cause cancer and changes in the genetic makeup and may damage the genome materials or disrupt cellular metabolic processes of exposed fish to humans that consume them (Isioma et al., 2017). There is greater need for further investigation since this approach may provide overestimation of cancer and mutagen potency of individual PAH, as most PAH indicated less comparative carcinogen than BaP.

CONCLUSION

Petrol and kerosene (Lighter petroleum products) with lower total mean ∑ 16PAH showed greater toxicity on exposed fish than crude and diesel oils (heavier products) with higher total mean ∑ 16PAH due to greater level of BaA to BaP compared to less toxic but carcinogenic DahA- BaP. On the other hand, heavier petroleum with greater total mean ∑ 8PAH were more carcinogenic and mutagenic compared to lighter petroleum with lowe total mean ∑ 8PAH. Further investigations are required since over estimation of cancer and mutagen potencies may not be entirely ruled out, considering the gap in BaP carcinogenic and mutagenic equivalent potencies of individual PAH to BaP.

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